

and/or skin roughness of the tissue. In some embodiments, the subject has loose or sagging skin. In some embodiments, the method improves roughness of skin. In some embodiments, the method restores volume moisture to the tissue. In some embodiments, the method results in reduced inflammatory response of the tissue. In some embodiments, the subject is in need of tissue repair. In some embodiments, the subject is suffering from a burn wound. In some embodiments, the method leads to healing of the tissue. In some embodiments, the skin is protected from free radical damage. In some embodiments, the composition supports epidermal cell-cell adhesion. In some embodiments, the composition supports a dermal epidermal junction. In some embodiments, the composition supports stem cells function and proliferation. In some embodiments, the composition supports intercellular communication. In some embodiments, the composition supports cellular recycling and protein homeostasis. In some embodiments, the composition prevents cellular senescence. In some embodiments, the composition supports collagen, elastin and other ECM components. In some embodiments, the composition supports heparan sulfate and proteoglycans.

DETAILED DESCRIPTION

[0016] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0017] Where the definition of terms as used in the specification departs from the commonly used meaning of the term, applicant intends to utilize the definitions provided herein, unless specifically indicated.

[0018] Treating soft tissue defects that may arise from aging has proven to be quite challenging. A number of materials have been used to correct soft tissue defects with varying degrees of success, however, no material has been found to be completely safe and effective. For example, silicon causes a variety of physiological and clinical problems including long term side effects, such as nodules, recurring cellulitis and skin ulcers.

[0019] Aging leads to defects including but not limited to fine lines and deep wrinkles, hyperpigmentation, elastin degradation, collagen degradation, inflammation, and decreased fibroblast function.

[0020] In some embodiments, a method for making compositions that include one or more proteins that are expressed during hypoxic conditions is provided. These proteins may be embryonic proteins, for example. In particular, the compositions are generated by culturing cells under hypoxic conditions on a surface (e.g., two-dimensional or three-dimensional) in a suitable growth medium. The culturing method produces both soluble and non-soluble fractions which may be used separately or in combination to obtain physiologically acceptable compositions having a variety of applications. The hypoxic conditions may induce proteins that stimulate precursor cells and may further strengthen the skin rejuvenating benefits of human fibroblast-derived growth factors.

[0021] In certain embodiments, a composition for repair and regeneration of skin tissue on a subject is disclosed comprising a conditioned medium collected from culturing human fibroblast cells under hypoxic conditions in a suitable cell culture medium and at least one additive, wherein the conditioned medium is stored in a first container and the additive is stored in a second container, and wherein the

conditioned medium and the additive are mixed prior to application to the skin tissue. The first container and the second container are separate chambers in a dual chamber container.

[0022] In certain embodiments, the human fibroblast cells are cultured under hypoxic conditions on a substrate grown in a suitable cell culture medium under 1-5% oxygen thereby producing embryo-like properties. The substrate may be microcarrier beads or a three-dimensional surface.

[0023] The compositions of the present invention may be colorless and further comprise at least one botanical or botanical extract in a range of about 0.5 to about 2.0% by weight of the composition, at least one peptide present within a range of about 0.0001 to about 0.001% by weight of the composition, at least one seed extract present within a range of about 0.5 to about 2% by weight of the composition, at least one marine extract within a range of about 0.01 to about 0.1% by weight of the composition, at least one bacterial ferment present within a range of about 0.5 to about 3.0% by weight of the composition, stem cell factors present within a range of about 0.1 to about 30% by weight of the composition, and/or cytokines.

[0024] In some embodiments, the composition may include cellular cytokines and growth factors. Extracellular proteins may be secreted into conditioned cell media such as growth factors, cytokines, and stress proteins. These may be used in the preparation of products for use in a large variety of areas including tissue repair, e.g., in the treatment of wounds and other tissue defects such as cosmetic defects as well as human and animal feed supplements. For example, growth factors are known to play an important role in the wound healing process. In general, it is thought desirable in the treatment of wounds to enhance the supply of growth factors by direct addition of these factors.

[0025] Cellular cytokines and growth factors are involved in a number of critical cellular processes including cell proliferation, adhesion, morphologic appearance, differentiation, migration, inflammatory responses, angiogenesis, and cell death. Studies have demonstrated that hypoxic stress and injury to cells induce responses including increased levels of mRNA and proteins corresponding to growth factors such as PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), and IGF (insulin-like growth factor) (Gonzalez-Rubio, M. et al., 1996, *Kidney Int* 50(1):164-73; Abramovitch, R. et al., 1997, *Int J. Exp. Pathol.* 78(2):57-70; Stein, I. et al., 1995, *Mol Cell Biol.* 15(10):5363-8; Yang, W. et al., 1997, *FEBS Lett.* 403(2):139-42; West, N. R. et al., 1995, *J. Neurosci. Res.* 40(5):647-59).

[0026] The growth factors are naturally secreted proteins which may regulate a variety of cell functions.

[0027] In some embodiments, the composition includes proteins that stimulate precursor cells.

[0028] The compositions of the present invention have a variety of applications including, but not limited to, promoting repair and/or regeneration of damaged cells or tissues, use in patches and implants to promote tissue regeneration (e.g., hernial repair, pelvic floor repair, rotator cuff repair, and wound repair), use in tissue culture systems for culturing cells, such as stem cells, use in surface coatings used in association with implantable devices (e.g., pacemakers, stents, stent grafts, vascular prostheses, heart valves, shunts, drug delivery ports or catheters, hernial and pelvic floor repair patches), promoting soft tissue repair, augmentation,